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Expression of Apoptotic Factors in Vaginal Tissues From Women With Urogenital Prolapse

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Abstract

Aims—Increased apoptotic activity in pelvic tissues may contribute to development of pelvic floor disorders. We evaluated expression of apoptotic factors (Bcl-2 family) in vaginal tissues from women with pelvic organ prolapse (POP) and how these factors correlate with severity of prolapse.

Methods—mRNA and protein expression of anti-apoptotic and pro-apoptotic factors in vaginal tissues from subjects and controls were determined by real-time PCR and Western blot. Severity of prolapse was staged using POP-Q criteria.

Results—Differential expression of Bcl-2 family factors was observed in protein rather than in gene expression. During the secretory phase, the anti-apoptotic (Bcl-2, Bcl-xl) and pro-apoptotic protein (Bax) were upregulated in controls compared to cases ($P < 0.05$). The ratios of Bcl-2/Bax and Bcl-2/Bad, which determine cellular sensitivity to induction of apoptosis, were higher in controls versus cases. Higher ratios indicate reduced cellular sensitivity to apoptosis. Protein expression of Bax and Bad was higher in women with severe compared to mild prolapse ($P < 0.05$).

Conclusion—Increased expression of Bad, Bax, and decreased ratios of Bcl-2/Bax, Bcl-2/Bad suggest increased apoptotic activity or sensitivity to induction of apoptosis in vaginal tissues of women with POP.

Keywords

apoptosis; Bcl-2 family proteins; pelvic floor disorders; pelvic organ prolapse

INTRODUCTION

Pelvic organ prolapse (POP) and urinary incontinence (UI) are common debilitating problems in adult women. The prevalence of both of these disorders increases with age and parity.¹ Depending on the study, the prevalence of prolapse has been estimated to be between 23.5% and 49.4% and that of UI to be 17.2% and 49.6%.² Mechanical strength of the pelvic tissues is essential for normal pelvic floor function. The vaginal wall prevents

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urethral and bladder neck descent by acting as the main support tissue of the urethra and bladder neck,^{3,4} while pelvic connective tissue support the uterus and upper vagina. Various types of abnormalities have been documented in the vaginal wall and connective tissue from women with urogenital prolapse, including increased proteolytic activity and apoptotic index⁵ in the extracellular matrix (ECM). Others have also observed a progressive and age-dependent occurrence of apoptosis of striated muscle cells in the urethral rhabdosphincter.⁶ These data suggest that apoptosis may be a contributor in the development of pelvic floor disorders.

Apoptosis, or programmed cell death, is fundamental to many physiologic processes, such as embryogenesis and tissue remodeling during healing.⁷ Thus, increased apoptosis may cause abnormal tissue remodeling after childbirth related pelvic trauma, pelvic surgeries, or even chronic mechanical stress due to weight bearing. Apoptotic cell death can be initiated by at least three major pathways. These pathways are regulated by (i) the death receptors, (ii) the mitochondria, and (iii) the endoplasmic reticulum. In most cells, the Bcl-2 family of proteins plays a critical role in the mitochondrial pathway. The Bcl-2 family is characterized by the presence of at least one of the four Bcl-2 homology (BH 1–4) domains. Pro-apoptotic proteins are subdivided into multi-domain (BH1-3; Bax) and BH 3-only domain (Bad). Anti-apoptotic proteins, Bcl-2 and Bcl-xl, bear three or four BH domains. During apoptosis, the Bcl-2 family pro-apoptotic proteins translocate to the outer membrane of mitochondria, promote the release of pro-apoptotic factors and induce apoptosis. The Bcl-2 family anti-apoptotic proteins prevent cell apoptosis by inhibiting the release of the pro-apoptotic factors in the mitochondria.^{8,9} Consequently, the balance between anti-apoptotic proteins and pro-apoptotic is critical to the ultimate apoptotic rate.¹⁰ Currently there is no data on these mechanisms in tissues from women with pelvic floor dysfunction.

We hypothesize that a chronic imbalance between anti-apoptotic proteins (Bcl-2, Bcl-xl) and pro-apoptotic proteins (Bax, Bad) in vaginal wall tissues contributes to the eventual development of pelvic floor disorders since it increases cellular sensitivity to apoptosis. Our primary goal in this pilot study is to examine whether Bcl-2 family members are increased in vaginal tissues of women with POP (cases) compared to controls. Our secondary objectives are to examine whether the Bcl-2 family protein expression changes throughout the menstrual cycle because apoptosis is modulated by ovarian hormones^{11,12} and to explore whether apoptotic activity correlates with severity of prolapse.

MATERIALS AND METHODS

Patient Selection and Tissue Collection

The Institutional Review Board of the Stanford University School of Medicine approved this study. Women with history of endometriosis, gynecologic malignancies, pelvic inflammatory conditions, connective tissue disorders, and prior pelvic surgery were excluded. Participants underwent a detailed pelvic examination, and the degree of prolapse was graded using the International Continence Society POP-Q criteria.¹¹ Continent women with no prolapse (stages <1) were entered in the control group. Menstrual phase was determined through endometrial histology confirmed with endometrial samples obtained at the time of surgery.

Vaginal wall tissues were collected as previously described.¹² Briefly, in women undergoing pelvic surgery, approximately 1 cm² of full-thickness, vaginal mucosa was excised 1 cm lateral to the urethrovaginal junction identified by a Foley balloon. Smaller, 0.5 cm² biopsies of vaginal mucosa from a similar area were excised from controls undergoing benign gynecologic surgeries. This area was chosen for biopsy since it is usually within the vagina or at the level of the hymen, therefore, relatively protected from external trauma. External trauma to the prolapsed tissues in stage 2–4 prolapse could artificially elevate apoptotic activity.

At the time of tissue collection, the epithelial layer was removed with a razor blade and confirmed by histology as described previously.^{13,14} The vaginal wall tissues used for determining mRNA and protein expression were frozen immediately in liquid nitrogen and then stored at –80°C for further processing.

Relative Quantification Real-Time PCR

Bcl-2, Bcl-xl, Bad and Bax mRNA expression levels were analyzed by quantitative real-time PCR (QPCR). The extraction of RNA from the tissue samples was carried out with the RNA-STAT-60 reagent. The cDNA was generated from 1 µg of total RNA, as described previously.¹⁵ PCR primers of Bax, Bcl-2 and Bcl-xl,¹⁶ Bad¹⁷ were used as described. QPCR was carried on the Mx3005P Multiplex Quantificative PCR System with MxPro QPCR software (Stratagene, La Jolla, CA). Brilliant SYBR Green QPCR Master Mix (Stratagene,) was used to perform PCR. QPCR was performed as described previously.¹⁴ The amplifications were done following a 10-min hot start at 95°C in a three-step protocol with 30 sec denaturation (94°C), 1 min annealing (55°C), and extension at 72°C for 30 sec. All PCR reactions were performed in duplicate. Forty cycles were performed. Hypoxanthine phosphoribosyl-transferase 1 (HPRT1) was used as an endogenous reference against which the different template values (Bcl-2, Bcl-xl, Bad and Bax) were normalized. Cycle of threshold (Ct) methods was used for quantification. Relative quantification of the gene of Bcl-2, Bcl-xl, Bad and Bax—corrected for the quantity of the normalizer gene (HPRT1)—was divided by one normalized control sample value (calibrator sample) to generate the relative quantification to calibrator (Rel. Quant. to Cal.). The calculations were done by MxPro QPCR software.

To allow for direct comparisons with minimal methodological variation, samples from vaginal tissues of controls and cases from the same phase of the menstrual cycle were processed together. We were only able to process 24 samples (two groups) at a time for one gene QPCR because the Mx3005P Multiplex Quantificative PCR System only has a 96-well blot. Comparisons between two groups were only made for the groups processed at the same time in order to minimize error. We did not pool data from different PCR runs for the data analysis.

Western Blot Analysis

Protein extraction from vaginal tissues with radio-immuno-precipitation assay (RIPA) buffer was performed as described previously.¹⁵ Total protein concentrations were determined using the Bradford method (Bio-Rad, Hercules, CA). One hundred micrograms of total

protein was separated by 10% polyacrylamide gels (SDS-PAGE). The gels were blotted onto nitrocellulose membranes (Pierce, Rockford, IL) in an electrophoretic transfer cell (Bio-Rad) and subjected to immunoblotting with antibody. Blots were blocked with 5% non-fat milk at room temperature for 2 hr, then probed with rabbit anti-Bcl-2 polyclonal antibody (1/500,) or rabbit anti-Bcl-xl polyclonal antibody (1/500) or rabbit anti-Bad monoclonal antibody (1/500) or rabbit anti-Bax polyclonal antibody (1/200, Abcam, Inc., Cambridge, MA) at 4°C overnight. After washing three times with phosphate buffered saline with 0.1% Tween, pH 7.4 (PBS-T), the membrane was incubated in a 1:5,000 dilution of donkey anti-rabbit IgG conjugated to HRP (GE Healthcare, Sunnyvale, CA) for 1 hr at room temperature. This was followed by three washes in PBS-T. Blots were developed by chemiluminescence. GAPDH was used as an internal loading control. HT1080 cell lysates (human fibrosarcoma) were used as positive controls. The band density was determined by Bio-Rad Quality One Software (Bio-Rad). To allow for direct comparisons with no gel-to-gel variation, samples from vaginal tissues of controls and cases from the same phase of the menstrual cycle were processed together on the same gel. Due to the size of the gel, we were only able to process 18 samples (two groups) at a time for each target protein. All Western blots were done in duplicates to assure that the differential expression detected was consistent. Data were analyzed per gel rather than pooled from different gels. To calculate the ratio of anti-apoptotic to pro-apoptotic proteins, each gel was re-probed with the respective Bcl-2 family protein.

Statistical Analysis and Sample Size

T-test was used to determine whether there was a statistically significant difference in Bcl-2 family members between the means of the cases and the controls processed on the same Western blot gel or in the same PCR run. The level of significance was set at $P < 0.05$. JMPIN software version 5.1 was used (SAS Institute, Inc., Cary, NC). Due to the lack of published data on Bcl-2 pathways in pelvic tissues and the fact that this is a pilot study, we estimated our sample size using a study examining apoptosis in the uterosacral ligament using the TUNEL method by Takacs et al.⁵

RESULTS

We recruited 36 premenopausal participants: 9 with POP (stage 2.3 ± 0.52) and 9 controls in the proliferative phase, and 9 with POP (stage 2.3 ± 0.82), and 9 controls in the secretory phase of the menstrual cycle (Table I). There were no significant differences in mean age and in number of vaginal deliveries between case and control groups in either phase of the menstrual cycle, although the control groups tended to have lower parity. To examine correlation with severity of prolapse (without the effect of hormones), we also recruited six age-matched menopausal women with mild prolapse (no prolapse beyond the introitus, stage 1.3 ± 0.52 , age 57.0 ± 5.4) and six menopausal women with severe prolapse (stage 3.0 ± 0.1 , age 57.3 ± 7.0). Parity was significantly higher in the severe prolapse compared to the mild prolapse group ($P = 0.009$).

mRNA Expression Levels of Bcl-2 Family Members in Vaginal Tissues

The mRNA expression level of the anti-apoptotic protein Bcl-2 in the control group ($N = 9$) was not significantly different from that in cases ($N = 9$) during the proliferative phase ($P = 0.67$). However, Bcl-2 mRNA expression in the control group ($N = 8$) was about four times higher than that in the POP group ($N = 8$) during the secretory phase ($P = 0.03$) (Table II).

The mRNA expression level of anti-apoptotic protein Bcl-xl and pro-apoptotic proteins Bad and Bax in the control group was similar to that in the cases in both the proliferative phase and secretory phase (Table II).

To examine the in vivo ability of reproductive hormones to modulate mRNA expression levels of Bcl-2, Bcl-xl, Bad and Bax in vaginal tissue, we compared the expression levels of these members between the proliferative and the secretory phases of the menstrual cycle for each group. The mRNA expression levels of the Bcl-2 family members did not change significantly throughout the menstrual cycle. This held true for both controls and cases. Although the mRNA expression of Bcl-2 in the secretory phase was about two times lower than that in the proliferative phase in the control group, it was not statistically significant (Table III).

Protein Expression Levels of Bcl-2 Family Members in Vaginal Tissues

During the proliferative phase, protein expression level of Bad in vaginal wall tissues from the control group ($N = 9$) was higher than those in the cases ($N = 8$) ($P = 0.03$, data not shown). Protein expression levels of Bax, Bcl-2 and Bcl-xl were similar in controls compared to cases (data not shown). During the secretory phase, the protein expression levels of Bax ($P = 0.04$), Bcl-2 ($P = 0.0001$), and Bcl-xl ($P = 0.04$) in vaginal tissues from the controls ($N = 9$) were all higher than those in cases ($N = 9$). Protein expression level of Bad in the controls was not significantly higher than that in cases (Fig. 1).

When we examined whether Bcl-2 family members are affected by the different phases of the menstrual cycle, Bax, Bcl-2 and Bcl-xl protein expressions did not change throughout the menstrual cycle in cases (Table IV). Bad, Bax and Bcl-xl protein expressions did not change throughout the menstrual cycle in controls (Table IV). However, Bad protein expression in cases was significantly higher in the secretory phase compared to the proliferative phase ($P < 0.05$, Table IV). Bcl-2 protein expression in the controls was significantly higher in the proliferative phase compared to the secretory phase ($P < 0.05$, Table IV). The discrepancy between these protein data and the above mRNA expression data indicate that differential expression of the Bcl-2 family members occurs at the protein level rather than mRNA level and, the apoptotic protein expression profile is different in prolapse versus control women.

Correlation Between Apoptotic Activity and Severity of Prolapse

To examine whether apoptotic activity correlates with severity of prolapse, we recruited 12 menopausal participants with POP: 6 with mild prolapse (stage 1–2, with no prolapse beyond the introitus, age 57 ± 2.2 , parity 1.8 ± 0.5) and 6 with severe prolapse (stage 3–4, age 57 ± 2.8 , parity 3.8 ± 3.0 ($P = 0.009$)). The effect of reproductive hormones was

eliminated by using only menopausal women not on hormones for this part of the study. We compared the protein expression levels of Bcl-2 family in the menopausal women with mild POP to the menopausal women with severe POP.

Although the protein expression levels of the anti-apoptotic proteins, Bcl-2 and Bcl-xl, did not show a difference between women with mild prolapse and women with severe prolapse, the pro-apoptotic proteins, Bad ($P = 0.0008$) and Bax ($P = 0.01$), expression levels were higher in the severe prolapse group compared to the mild prolapse group (Fig. 2). These findings are consistent with increased pro-apoptotic environment in tissues from women with severe prolapse.

The Ratio of Anti-Apoptotic Proteins (Bcl-2 and Bcl-xl) to Pro-Apoptotic Proteins (Bax and Bad) in Vaginal Tissues

Since the ratio of anti-apoptotic proteins to pro-apoptotic proteins is a determinant of cellular sensitivity to apoptosis, we also examined differences in these ratios. Higher ratios indicate lower sensitivity to apoptosis. In the premenopausal groups, the ratios of Bcl-2/Bax, Bcl-2/Bad, Bcl-xl/Bax, and Bcl-xl/Bad were similar in the control group compared to the cases during the proliferative phase (data not shown). During the secretory phase, the ratios of Bcl-2/Bax ($P = 0.0004$) and Bcl-2/Bad ($P = 0.006$) of protein expression level in vaginal wall tissues from the controls ($N = 9$) were significantly higher than those in the cases ($N = 9$). This suggests that tissues from controls are less sensitive to induction of apoptosis compared to those from women with POP. The ratios of Bcl-xl/Bax and Bcl-xl/Bad were similar in the controls compared to cases (Fig. 3). In the menopausal groups, the ratios of Bcl-2/Bax, Bcl-2/Bad, Bcl-xl/Bax, and Bcl-xl/Bad were similar in the women with mild POP compared to the women with severe POP (data not shown).

DISCUSSION

Apoptosis is important in tissue remodeling during repair and healing processes. Because the vaginal wall tissues suffer cumulative mechanical stresses from daily weight-bearing activities and significant trauma during vaginal delivery, small differences in the rate of apoptosis throughout decades may contribute to abnormally repaired tissues decades later leading to eventual development of pelvic floor disorders. Takacs et al.¹⁸ have documented increased apoptotic index using TUNEL staining of apoptotic cells in vaginal tissues from women with prolapse compared to controls. The TUNEL methodology provides a global apoptosis rate but does not specify which apoptotic pathway is upregulated. Currently there is no data on specific apoptotic mechanisms in vaginal tissues from women with POP. In this pilot study we sought to further investigate the specific factors involved in this increased apoptotic activity.

Bcl-2 family proteins regulate cell apoptosis through the intrinsic mitochondrial pathway. They consist of pro-apoptotic (group II and III proteins, such as Bax, Bad) and anti-apoptotic (group I proteins, such as Bcl-2, Bcl-xl) members. Both the expression level of these proteins and the ratio of pro-apoptotic to anti-apoptotic members are important determinants of cellular sensitivity to induction of apoptosis.¹⁹ We compared the expression levels of the anti-apoptotic Bcl-2, Bcl-xl and pro-apoptotic Bax, Bad proteins in vaginal wall

tissues of age-matched control premenopausal women to women with POP to examine whether proteins involved in apoptosis are differentially expressed. Because ovarian hormones are known to modulate apoptosis, we also examined whether there is differential expression of Bcl-2 family members throughout the menstrual cycle.

We found that the changes in the expression of Bcl-2 family members in vaginal wall tissues mainly occurred at the post-translation level rather than at the mRNA level. We observed significant increases in Bcl-2 protein expression in vaginal wall tissues during the proliferative phase compared to the secretory phase in controls, and in Bad protein expression during the secretory phase compared to the proliferative phase in the women with POP (Table IV). This suggests that Bcl-2 family protein expression in vaginal wall tissues may be regulated by reproductive hormones, with tissues from women with prolapse expressing a different Bcl-2 protein profile compared to tissues from controls. This finding is consistent with the observed increase in apoptosis in the human endometrium at the end of the secretory phase.^{20,21} Estrogen down-regulates Bax expression in the medial preoptic nucleus of the rat²² and progesterone decreases Bcl-2 expression in human endometrium.²³ Higher Bax expression—as a result of low estrogen and high progesterone levels during the secretory phase—may induce apoptotic activity in vaginal wall tissues as well as in the endometrium during the secretory phase.

Liu et al.²⁴ documented that the overall growth rate of POP fibroblasts compared to that of the controls was significantly slower and suggested that slow fibroblast turnover may contribute to the development of POP. In the present study we found that the protein expression levels of Bax, Bcl-2 and Bcl-xl were significantly higher in controls compared to cases in the secretory phase (Fig. 1). The over-expression of the anti-apoptotic proteins Bcl-2 and Bcl-xl in controls may counteract the concurrent increase in pro-apoptotic protein, Bax, during tissue remodeling in the vaginal wall tissues, thus resulting in a neutral or anti-apoptotic effect in controls. Consistent with this, the ratios of anti-apoptotic protein to pro-apoptotic proteins, Bcl-2/Bax and Bcl-2/Bax, were higher in the controls compared to women with POP. Conversely, this implies that the vaginal tissues from women with POP exhibit higher apoptotic activity or susceptibility to apoptosis compared to tissue from controls.

In this study we also compared the expression level of Bcl-2 family proteins of age-matched menopausal women with mild POP (stage 1–2) with that of menopausal women with severe POP (stage 3–4) to assess whether their apoptotic activity correlated with severity of prolapse. Menopausal patients were used to eliminate the effect of reproductive hormones. We observed a higher expression of pro-apoptotic proteins Bad and Bax in the menopausal women with severe POP compared with the women with mild POP. However, the ratios of anti-apoptotic proteins (Bcl-2, Bcl-xl) to pro-apoptotic proteins (Bad, Bax) were not significantly different between the two groups. This may be due to the small sample size of these comparisons.

The observed lower ratios of anti-apoptotic to pro-apoptotic proteins in the vaginal wall tissues from the women with POP compared to controls and increased Bad and Bax with severity of prolapse are consistent with increased apoptotic activity and susceptibility to apoptosis in women with prolapse. This chronic, low-grade apoptotic environment can

ultimately contribute to reduced function of the pelvic supportive tissues with the resultant clinical manifestation or worsening of POP. Limitations to our study include small sample size and a study design that does not allow us to determine cause-or-effect. Given the small sample size, we attempted to minimize error related to methodology by processing the comparison groups in the same PCR run or on the same Western blot. Furthermore, statistical comparisons were only made for the two groups that were processed together. We did not pool data from different PCR runs or gels. Nevertheless, larger studies with appropriate sample sizes based on these pilot data are needed to confirm our observations. Although we took biopsies at the urethrovesical junction to minimize inflammatory effect, it is possible that there is differential gene expression due to the tissue proximity to the leading edge of the prolapse. Our study provides insight into the ongoing apoptotic mechanisms and environment in pelvic tissues from women with prolapse. Future investigations on which pathways are activated during injury could lead to design of treatment or preventive options.

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Fig. 1.

Western blotting of Bad, Bax, Bcl-2, and Bcl-xl in vaginal wall tissues from women with POP (n = 9) and controls (n = 9) in the secretory phase. “S” represents women with POP/SUI. “C” represents controls. The bars represent the mean ± SEM. The protein expression levels were normalized with GAPDH expression level. Densitometric analysis showed that the protein expression levels of Bax ($P < 0.001$), Bcl-2 ($P = 0.003$), and Bcl-xl ($P = 0.03$) were higher in the control group (n = 9) than in the POP/SUI (n = 9, $P = 0.03$). @, #, \$ $P < 0.05$, when the control group was compared with the POP/SUI.

Fig. 2.

Western blot comparison of Bad, Bax, Bcl-2, and Bcl-xl protein expression levels in vaginal wall tissue from menopausal women with mild prolapse (lane 1–6) and severe prolapse (lane 7–12). The numbers represent the mean $\pm$ SEM. The protein expression levels were normalized with GAPDH expression level. The protein expression levels of Bad ($P < 0.0001$) and Bax ($P = 0.02$) in the women with severe POP ($n = 6$) were higher than in the women with mild POP ($n = 6$). *, @ $P < 0.05$.

Fig. 3.

The protein expression ratios of Bcl-2/Bax, Bcl-2/Bad, Bcl-xl/Bax, and Bcl-xl/Bad were determined by Western blotting. During the secretory phase, the ratios of Bcl-2/Bax ($P = 0.0004$), Bcl-2/Bad ($P = 0.0006$) were significantly higher in the control group ($n = 9$) compared to POP ($n = 9$). *, @ $P < 0.05$, when the control group was compared with the POP/SUI.

TABLE I

Patient Demographics

	POP (mean ± SEM)		Controls (mean ± SEM)		P-values	
	Age (years)	Parity	Age (years)	Parity	Age	Parity
Menstrual phase						
Proliferative phase	41.3 ± 2.2	0–3	45.4 ± 1.6	1–2	0.12	0.13
Secretory phase	43.3 ± 1.4	0–4	42.4 ± 1.3	0–3	0.71	0.07

TABLE II

Relative Real-Time QPCR Was Performed to Compare the mRNA Expression Levels of Bcl-2 Family Members in Vaginal Wall Tissue of Women With POP and Controls From Different Menstrual Phases

Bcl-2 family gene	Proliferative phase			Secretory phase		
	POP (N = 9)	Control (N = 9)	P-value	POP (N = 8)	Control (N = 8)	P-value
Bad	1.24 ± 0.31	1.8 ± 0.54	0.34	3.96 ± 1.5	3.2 ± 0.76	0.63
Bax	25.3 ± 7.7	25.7 ± 6.4	0.96	1.94 ± 0.62	1.89 ± 0.53	0.94
Bcl-2	1.93 ± 0.61	2.3 ± 0.7	0.67	143.3 ± 62.27	717.75 ± 221.63	0.03*
Bcl-xl	2.9 ± 0.3	5.7 ± 1.5	0.13	3.9 ± 1.4	3.9 ± 0.68	0.99

The numbers (*) represent the mean ± SEM. mRNA expression levels of Bcl-2 was significantly higher in the control compared to POP group during the secretory phase ($P = 0.03$).

Comparison of Bad, Bax, Bcl-2, and Bcl-xl mRNA Expression Levels Between Different Menstrual Phases in vaginal Wall Tissue of Women With POP or Controls

TABLE III

Bcl-2 family gene	POP		Control		P-value
	Proliferative phase (N = 9)	Secretory phase (N = 9)	Proliferative phase	Secretory phase (N = 9)	
Bad	2.32 ± 0.70	3.33 ± 1.65	2.54 ± 0.67	2.9 ± 0.54	0.66
Bax	0.71 ± 0.17	0.57 ± 0.2	1.03 ± 0.33	0.82 ± 0.17	0.57
Bcl-2	1.52 ± 0.41	1.131.52 ± 0.4	0.361.52 ± 0.10	0.14 ± 0.04	0.06
Bcl-xl	1.97 ± 0.69	2.6 ± 1.04	4 ± 1.04	2.1 ± 0.33	0.11

mRNA expression of Bcl-2 family proteins did not change throughout the menstrual cycle in both the POP and control groups.

TABLE IV

Comparison of Bad, Bax, Bcl-2, and Bcl-xl Protein Expression Levels (Western Blot) Between Different Menstrual Phases in Vaginal Wall Tissue of Women With POP or Controls

Bcl-2 family protein	POP			Control		
	Proliferative phase (N = 9)	Secretory phase (N = 9)	P-value	Proliferative phase (N = 9)	Secretory phase (N = 9)	P-value
Bad	0.54 ± 0.02	0.56 ± 0.06	0.03*	0.28 ± 0.009	0.28 ± 0.013	0.93
Bax	0.42 ± 0.006	0.42 ± 0.007	0.92	0.51 ± 0.0078	0.51 ± 0.5	0.91
Bcl-2	0.61 ± 0.01	0.61 ± 0.01	0.98	1.26 ± 0.027	1.19 ± 0.01	0.015*
Bcl-xl	1.5 ± 0.044	1.4 ± 0.033	0.14	0.85 ± 0.03	0.93 ± 0.05	0.22

The protein expression levels were normalized with GAPDH expression level. The numbers represent the mean ± SEM. The protein expression levels of Bad ($P = 0.016$) in the POP group and Bax ($P = 0.0005$) in the control group were higher in the secretory phase ($n = 9$) than in the proliferative phase ($n = 9$). Bcl-2 and Bcl-xl protein expressions did not change throughout the menstrual cycle.

* $P < 0.05$.